

Docket No.: 05408/100J111-US2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Michael Ludensky et al.

Application No.: Not Yet Assigned

Confirmation No.: N/A

Filed: Concurrently Herewith

Art Unit: N/A

For: METHOD FOR REMOVAL OF BIOFILM

Examiner: Not Yet Assigned

CLAIM FOR PRIORITY AND SUBMISSION OF DOCUMENTS

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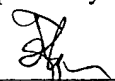
Applicant hereby claims priority under 35 U.S.C. 119 based on the following prior applications filed in the United States Patent and Trademark Office on the date indicated:

<u>Application No.</u>	<u>Date</u>
60/435,680	December 20, 2002

A certified copy of the aforesaid Patent Application was received by the International Bureau on July 17, 2003, during the pendency of International Application No. PCT/US03/017882. A copy of Form PCT/IB/304 is enclosed.

Dated: June 20, 2005

Respectfully submitted,

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APPLICATION NUMBER: 60/435,680

FILING DATE: December 20, 2002

RELATED PCT APPLICATION NUMBER: PCT/US03/17882

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PATENT TRADEMARK OFFICE

Docket No: 5408/OJ111

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805 Third Avenue
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Date: December 20, 2002

Box Provisional-Application
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Enclosed please find a provisional application for United States patent as identified below:

Inventor/s (ALL inventors, including NAME, plus city and state of RESIDENCE for each):

Michael L. LUDENSKY, Randolph, New Jersey
Philip G. SWEENEY, Hackettstown, New Jersey

Title: METHOD FOR REMOVAL OF BIOFILM

including the items indicated:

1. Specification and 10 claims: 1 indep.; 9 dep.; _ multiple dep.
2. [X] Drawings, 5 sheets (Fig. 1-5)

PROVISIONAL PATENT APPLICATION COVER SHEET

3. Unexecuted Declaration and Power of Attorney
4. ☒ Check in the amount of \$160.00, (\$160 filing; \$0 recording)

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Docket No. 5408/OJ111

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PATENT TRADEMARK OFFICE

Docket No.: 5408/0J111

METHOD FOR REMOVAL OF BIOFILM**Background of the Invention**

5 Biofilm may be defined as an undesirable accumulation of microorganisms on a surface. It is estimated that more than 99% of all the planet's bacteria live in biofilm communities. Biofilm consists of cells immobilized in a substratum, frequently embedded in an organic polymer matrix of microbial origin, which can restrict the diffusion of substances and bind antimicrobials. In flowing aquatic environments, a biofilm consists of a sticky and

10 absorptive polysaccharide matrix encompassing microorganisms. Biofilm bacteria are morphologically and metabolically distinct from free-floating bacteria. Their structural organization is a characteristic feature and distinguishes biofilm cultures from conventional planktonic organisms.

15 Biofilms create problems for industry from corroding water pipes to computer-chip malfunctions. Any man-made device immersed in an aquatic habitat is susceptible to colonization by microbial biofilm. For example, biofilm may be present on the surfaces of ship bottoms, industrial pipelines, household drains, and artificial hip joints. For the industrial manufacturer, biofilm clusters represent a source of microbial inoculation in a system and may cause plugging problems. In addition to their role as fouling agents, biofilms may also have

20 adverse effects on people, including altering their resistance to antibiotics and affecting the immune system. Thus, there exists a need in the art for developing effective methods of removing biofilm.

The dynamic nature of biofilms makes it difficult to measure and monitor biofouling. Biofilms often include embedded inorganic particles such as sediments, scale deposits, and corrosion deposits. Moreover, biofilms continuously change in thickness, surface distribution, microbial populations and chemical composition, and respond to changes in environmental factors such as water temperature, water chemistry and surface conditions. Thus, the complexity of biofilms has reduced the effectiveness of treatment and removal strategies.

Even though most microorganisms in industrial systems are associated with biofilm, they have historically received less attention than planktonic microorganisms. However, it has been shown that various biocides are less effective against biofilm than dispersed cells of the same organism. The most common biocides used in biofilm control are pure free halogen donors such as NaOCl and NaOCl/NaOBr. These, however, must be used in high quantities to be effective. In addition, several recent studies evaluating halogen efficacy on biofilms showed an increased disinfection resistance of attached bacteria to free chlorine. Free chlorine treatment at concentrations usually effective against planktonic microorganisms have little effect on the number of attached bacteria or on their metabolic activity. The data indicate that the transport of free chlorine into the biofilm is a major rate-limiting factor, and increasing concentrations did not increase biocidal efficiency. Griebe, T., Chen, C.I., Srinavasan, R., Stewart P., "Analysis of Biofilm Disinfection By Monochloramine and Free Chlorine, *Biofouling and Biocorrosion In Industrial Water Systems*, (edited by G. Geesey, Z. Lewandowski, and H-C. Flemming), pp.151-161, Lewis Publishers (1994).

Excessive reactivity of pure free halogen donors was overcome by using bromochlorodimethylhydantoin (BCDMH). The published study by M. Ludyansky and F. Himpler entitled "The Effect of Halogenated Hydantoins on Biofilms," NACE, paper 405 (1997), demonstrated higher efficacy on biofilms compared to pure free halogen donors. However, while effective, it is still not an efficient halogen source when applied to biofilm.

Others have attempted to suppress biofilm growth in aquatic systems by using an oxidizing halogen with the addition of adjuvant. U.S. Pat. No. 4,976,874 to Gannon et al., incorporated herein by reference, discloses a method and formulation for the control of

biofouling using an oxidizing halogen in combination with a non-oxidizing quaternary ammonium halide. However, this method poses environmental issues.

Thus, the control of biofilm in aquatic systems has typically involved the addition of oxidizing and non-oxidizing biocides to bulk water flow. However, high levels of these expensive chemicals are needed because their effectiveness is rapidly reduced as a result of exposure to the various physical and chemical conditions in specific applications since the concentration of the biocides is considerably reduced by the time the biocides reach the biofilm.

Summary of the Invention

The present invention is directed to a method of removing biofilm from an aqueous medium. The method comprises adding one or more chlorinated hydantoins, specifically, monochlorodimethylhydantoin (MCDMH) or dichlorodimethylhydantoin (DCDMH), to the aqueous medium. The concentration of the chlorinated hydantoins maintained in the aqueous medium generally ranges from about 0.01 to about 100 ppm (expressed as Cl_2) for biofilm inhibition.

In a concentrate, the concentration of the chlorinated hydantoins generally ranges from about 0.1 up to 50% by weight based on the total weight.

The present invention has application to essentially all aqueous systems containing or having the potential to contain biofilm. These may be cooling water, pulping or papermaking systems, and air washer systems, as well as agricultural potable and drainage systems, food preparation and cleaning systems, brewery, dairy and meat-producing systems, and oil industry systems. Aqueous systems also include any potable water systems, including drinking water systems, as well as recreational water systems, such as swimming pools and spas; household water-related systems, including toilet bowls, drains, sewers, shower stalls, bathtubs, and sinks, as well as institutional "water-related" systems, hospital systems, and dental water systems and any system where a medical device is in contact with an aquatic medium.

Brief Description of the Figure

Figure 1 illustrates the effect of NaOCl on heat transfer resistance (HTR) which correlates to biofilm formation and accumulation and dissolved oxygen (DO) level in an aqueous system.

5 Figure 2 illustrates the effect of NaOBr on heat transfer resistance (HTR) and dissolved oxygen (DO) level in an aqueous system.

Figure 3 illustrates the effect of BCDMH/MEH on heat transfer resistance (HTR) and dissolved oxygen (DO) level in an aqueous system.

10 Figure 4 illustrates the effect of MCDMH on heat transfer resistance (HTR) and dissolved oxygen (DO) level in an aqueous system.

Figure 5 illustrates the effect of DCDMH on heat transfer resistance (HTR) and dissolved oxygen (DO) level in an aqueous system.

Detailed Description of The Invention

15 The extent and nature of biofilm removal, of course, varies with the context of the problem. The diverse nature of the problems and the diverse environments in which biofilms grow calls for a variety of tactics and strategies for biofilm removal. With respect to established biofilms, it is often desirable to remove it rather than to merely sterilize and leave it *in situ*. In addition, it may be important to kill the cells forming the biofilm and prevent them from
20 spreading to other locations. Thus, for purposes of the present invention, the term "removal" of biofilm includes the removal of existing biofilm and the prevention of biofilm microorganism regrowth in a treated system. This is a more difficult task than "biofilm control" which includes both the prevention of biofilm growth from a clean system and the prevention of continued growth in a treated system upon which biofilm has already formed.

25 The term "chlorinated hydantoin" refers to an hydantoin which may be in the form of a pure compound, such as, monochlorodimethylhydantoin or an admixture of hydantoins, i.e., monochlorodimethylhydantoin and dichlorodimethylhydantoin mixtures, or mixtures of hydantoins with degree of halogenation between 0.1 and 2.0.

The alkyl moieties of the chlorinated hydantoin may be the same or different, preferably alkyl groups having 1 to 6 carbon atoms and alkynyl groups having 2 to 6 carbon atoms.

Preferred chlorinated hydantoins include, but are not limited to, dichloro-5,5-dimethylhydantoin, monochloro-5,5-dimethylhydantoin (MCDMH), dichloro-5-methyl-5-ethylhydantoin, monochloro-5-methyl-5-ethylhydantoin, and any combination of any of the foregoing.

The mixture may be in the form of a solid, liquid, or a gas. The chlorinated hydantoin may be a solid. The term "solid" includes powders, granules, tablets, briquettes and slurries.

Concentrates of the chlorinated hydantoin may contain greater concentrations of active ingredient than typical biofilm control concentrates. For example, a solid concentrate of chlorinated hydantoin typically contains 70% by weight of active ingredient (expressed as Cl_2) based upon 100% total weight of concentrate. In contrast, liquid concentrates of sodium hypochlorite typically comprise only about 12% by weight of active ingredient based upon 100% total weight of concentrate. Additionally, the chlorinated hydantoins of the present invention are stable, unlike most bleaches currently sold.

While the above discussion refers to the treatment of an aqueous system containing biofilm with the chlorinated hydantoin, it is also contemplated that the aqueous system be formed after a dry biofilm or biofilm in a non-aqueous medium is brought in contact with a solid or granular halogenated hydantoin. In such instance, the aqueous system may be formed by the addition of water or water vapor to the two solids or water-free materials.

The amount of the chlorinated hydantoin added to the aqueous medium is sufficient to remove the biofilm. This is generally from about 0.01 to about 100 ppm (expressed as Cl_2), preferably from about 0.05 to about 25 ppm (expressed as Cl_2).

In addition to adding the pre-formed halogenated hydantoin to the aqueous system, it may be desirable to form the halogenated hydantoin *in situ*. This can be done by adding a di-substituted hydantoin and an halogenating agent to the biofilm containing aqueous system separately in the appropriate molar ratio. For example, an alkali metal hypochlorite (NaOCl) or

KOCl) and dimethylhydantoin can be added in a molar ratio sufficient to form the desired amount of the halohydantoin.

In some systems, such as cooling water systems, additives are always used. In other systems, such as swimming pools, there may be no performance additives.

5 Performance additives (i.e., compositions that enhance the quality and use of the chlorinated hydantoins) include, but are not limited to, cleaning agents, biodispersants, solubility modifiers, compaction aids, fillers, surfactants, dyes, fragrances, dispersants, lubricants, mold releasers, detergent builders, corrosion inhibitors, chelants, stabilizers, bromide sources, and scale control agents. An important requirement is that the material be compatible with the
10 chlorohydantoin composition.

Solubility modifiers which may be added to chlorinated hydantoins described herein include, for example, sodium bicarbonate, aluminum hydroxide, magnesium oxide, barium hydroxide, and sodium carbonate. See U.S. Patent No. 4,537,697. Solubility modifiers can be used in the compositions in an amount ranging from 0.01% to 50% by weight.

15 Examples of compaction aids are inorganic salts including lithium, sodium, potassium, magnesium, and calcium cations associated with carbonate, bicarbonate, borate, silicate, phosphate, percarbonate, and perphosphate. See U.S. Patent No. 4,677,130. Compaction aids can be used in the compositions in an amount ranging from 0.01% to 50% by weight.

20 Fillers which may be added to the chlorohydantoins include, for example, inorganic salts, such as lithium, sodium, potassium, magnesium and calcium cations with sulfate, and chloride anions, as well as other inorganics such as clays and zeolites. Fillers are used in compositions to reduce product costs and can be added in an amount ranging from 0.01% to 50% by weight.

25 The biodispersant enhances the efficacy of the chlorinated hydantoin as a biofilm control agent and assists in maintaining the surfaces of the container in which the aqueous medium is contained clean. They are typically surfactants and preferably surfactants with a non-biocidal effect on microorganisms and biofilms. Examples of biodispersants include Aerosol OTB (sodium dioctyl sulfosuccinate), disodium lauryl sulfosuccinate, sodium lauryl sulfoacetate,

as well as other sulfonates. Surfactants are used in the compositions to enhance cleaning performance and can be added in an amount ranging from 0.01% to 20% by weight. Generally, such a mixture contains from about 80% to about 99.99% by weight of chlorinated hydantoin and from about 0.01% to about 20% by weight of biodispersant, based upon 100% total weight of mixture; preferably, from about 90 to about 99.99% by weight of chlorinated hydantoin and from about 0.01% to about 10% by weight of biodispersant.

An aqueous solution of the desired non-chlorinated hydantoin(s) at the desired mole ratios may be prepared by the methods described in U.S. Patent No. 4,560,766, and Petterson, R. C., and Grzeskowiak, V. , J. Org. Chem., 24,1414 (1959) and Corral, R. A., and Orazi, O. O., J. Org. Chem., 28, 1100 (1963), both of which are hereby incorporated by reference.

EXAMPLE 1

Biofilm Inhibition Control Efficacy

The efficacy of biocides and biocides with dispersants was estimated by a reduction of biofilm dry weight in test flasks, compared to untreated controls. Biofilm development was determined gravimetrically by the methods described in Ludyansky, M., Colby, S., *A Laboratory Method for Evaluating Biocidal Efficacy on Biofilms*, Cooling Tower Institute, Paper TP96-07 (1996).

The sheathed *Sphaerotilus natans* (ATCC 15291), which is known to be very resistant to any chemical control and found in a variety of applications (cooling water systems, paper process waters, and sewage treatment processes), was used in the tests.

The bacteria were cultivated at 25-30° C in a 5% CGY medium which contained: 5g of casitone (Difco), 10g of glycerol, and 1g of yeast autolysate (Difco) per liter of DI water. The inocula contained approximately 10^6 cells per milliliter. 8 oz. flasks were filled with 150 ml of 5% CGY media and 1 ml of *Sphaerotilus natans* inoculum. The flasks were filled with the test biocides, namely, NaOCl, NaOBr, MCDMH. Additional flasks, not containing a biocide, served as controls. The flasks were installed on a shaker and maintained at 22-30°C rotating at 100-200 rpm for 48-72 hours. The contents were dried for 5 hours at 105°C and cooled

overnight. The difference between the weight of the flasks containing the dried biomass and the tare weight of the flasks represented the dry biofilm mass.

The effectiveness of biofilm prevention was calculated as a percent change in growth based on the difference between the average dried biofilm weight in the untreated controls and in the treated flasks, according to the following formula: $E\% = (B_{\text{control avg}} - B_{\text{avg}}) / B_{\text{control avg}} * 100$, where $E\%$ = percent reduction of biofilm growth, B = Biofilm weight, and B_{control} = Biofilm weight in the control flask.

The results of the experiments, including the concentration of the biocides, are set forth in Table 1:

TABLE 1

Biocide	Concentration, ppm	B avg, g	B control avg, g	E, %
NaOCl	10	0.0028	0.0185	84.86
NaOBr	10	0.0013	0.0185	92.97
MBDMH	10	0.0008	0.0185	95.7
MCDMH	10	0.0005	0.0185	97.3
DCDMH	10	0.0005	0.0173	97.1
NaOCl	5	0.009	0.0144	37.5
NaOBr	5	0.0021	0.0144	85.4
MBDMH	5	0.0081	0.0152	46.7
MCDMH	5	0.0007	0.0144	95.1
DCDMH	5	0.0009	0.0173	94.8

The results show that chlorinated hydantoin (MCDMH) was a superior biofilm inhibition agent over free halogen donors (NaOCl or NaOBr).

EXAMPLE 2

Biofilm Removal Control Efficacy

Sphaerotilus natans (ATCC 15291), as in Example 1 was used in the tests described below.

Biofilm test system

An on-line testing system for chlorinated biocide efficacy testing was used to provide a real-time, non-destructive method for biofilm monitoring and measurement. The

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system monitors the heat transfer resistance (HTR) which correlates to biofilm formation and accumulation, and dissolved oxygen (DO) level in the bulk water which correlates with changes in biofilm activity. The system design, parameters and growth conditions are disclosed in Ludensky, M., "An Automated System for Biocide Testing on Biofilms." *Journal of Industrial Microbiology and Biotechnology*, 20:109-115 (1998).

The system consisted of a continuous-flow heat-exchange loop, a biological growth reactor (chemostat) and subsystems for life support, biofilm measurement, and environmental control. All system parameters, including water flow, temperature, dilution rate and nutrient concentration, were optimized for obtaining fast, heavy and reproducible biofilm growth. The system make-up water was kept at constant oxygen saturation (by continuous sparging of air), temperature, and pH conditions. Thus, any changes in DO concentrations or pH levels in the recirculating water were considered due to biofilm activity. All monitoring and control parameters were calculated in the data acquisition system, which was controlled by a custom-designed computer software program. Data was collected every 15 seconds, with averages calculated and recorded every 3 to 60 minutes in a spreadsheet for subsequent graphical analysis. The program was designed so that the system was able to function continuously under constant conditions for several weeks. Biocide efficacy testing was conducted through analysis and comparison of the shape and values of the corresponding curves of HTR and DO. Analysis included consideration of curve patterns corresponding to biocide treatment, as well as biofilm recovery (regrowth).

Growth Conditions

The sheathed *Sphaerotilus natans* (ATCC 15291), known to form a tenacious biofilm on heat exchanger surfaces in cooling water systems and papermaking machines, was selected for biofilm growth. Inocula were pumped into the microbial growth reactor and allowed to sit at room temperature overnight. The next day, make-up water and nutrient (CGY media) were added. Selection of initial growth conditions and parameters of the system was based on previous experience, laboratory limitations, geometric size of the system's components, and the desire to promote a growth of biofilm. Shifting of growth conditions from planktonic growth to

attached filamentous growth was obtained by lowering media concentrations to less than 5% and maintaining dilution rates higher than maximum specific rate. Test conditions are shown in Table 2.

TABLE 2
Biofilm System On-Line Test Conditions

Parameter	Conditions
pH	7.2-8.5
Temp., circulating water	74-76° F
Temp., wall	85° F
Makeup water	Clinton tap
Substrate concentration	CGY; 30-70 ppm
Inoculum	S. natans
Water flow	3 fps
Dilution rate	0.9
Make up water	170 ml/min
Nutrient addition	1 ml/min
System volume	10 liters

5

Biocidal efficacy of the test solutions was determined by analysis of the shape of the HTR and DO curves indicating the biofilm's response to biocidal treatments.

10 Treatment Programs

During treatment programs, the system was continuously fed with nutrient and make-up water (constant chemistry, oxygen and temperature). Three modes of treatments were tested, namely, slug, slug plus continuous, and continuous.

15 Slug treatment was conducted by the addition of a prepared stock solution in a precalculated dose (per volume of the circulating water in the system) to the chemostat. In the slug plus continuous mode, biocide treatment was carried out by an initial slug dose injected to

overcome halogen demand, followed by a continuous, 3-hour treatment at a constant concentration based upon the makeup water rate.

Biocide Preparation and Monitoring

5 All five biocides, NaOCl, NaOBr, MCDMH, BCDMH/MEH, and DCDMH, were prepared as 1000 ppm fresh Cl₂ master solutions. Treatment concentrations for all biocides were calculated from the measurement of free and total residual halogen, as measured by the DPD Cl₂ test, conducted immediately before treatment.

10 Tests incorporating repeated slug plus continuous treatments at increasing initial concentrations (10, 15 and 20 ppm) were performed for three consecutive days on NaOCl, NaOBr, MCDMH, BCDMH/MEH, and DCDMH. Heat transfer rate and dissolved oxygen levels in the system were automatically monitored and their dynamics were analyzed. Based on obtained parameters, the following conclusions were reached:

15 NaOCl, NaOBr, and BCDMH/MEH were not able to remove biofilm at any of the tested concentrations. Biofilm recovery was observed 24 hours after the start of each treatment and HTR values were higher than values observed at the start of each treatment, as shown in Figs. 1, 2 and 3.

20 Dissolved oxygen response to biocide treatment was the strongest in the case of DCDMH, and the weakest in the case of NaOCl. Through analysis of curve patterns (Fig. 1 - Fig. 5), it was concluded that biofilm regret control could be achieved by a slug plus continuous treatment of 15 ppm BCDMH/MEH or 20 ppm of NaOBr as shown in Fig. 2 and Fig. 3. However, neither of these biocides was able to initiate biofilm removal.

25 Testing of chlorinated hydantoin MCDMH and DCDMH demonstrated a unique effect: biofilm sloughing occurred soon after addition of 20 ppm of either MCDMH or DCDMH. The results of the tests are shown in Fig. 4 and Fig. 5. This effect is not common for any other oxidizing biocides.

The observations set forth above are summarized in the following table:

TABLE 3
Slug Plus Continuous Treatment

	<u>BIOFILM CONTROL</u>		<u>BIOFILM REMOVAL</u>
	<u>HTR</u>	<u>DO</u>	<u>HTR</u>
NaOCl	Not effective	Weakest	No
NaOBr	Effective at 20 ppm	Moderate	No
BCDMH/MEH	Effective at 15 ppm	Moderate	No
MCDMH	Effective at 15 ppm	Moderate	Yes at 20 ppm
DCDMH	Effective at 15 ppm	Strongest	Yes at 20 ppm

5

What is claimed is:

- 1 1. A method of removing biofilm in an aqueous medium which comprises adding
2 one or more chlorinated hydantoins to the medium and the concentration of the chlorinated
3 hydantoin maintained in the medium is from about 0.01 to about 100 ppm (expressed as Cl₂).
- 1 2. The method of claim 1, wherein the chlorinated hydantoin is
2 monochlorodialkylhydantoin, dichlorodialkylhydantoin, monochlorodialkynylhydantoin,
3 dichlorodialkynylhydantoin or a mixture thereof, wherein the alkyl group contains from 1 to 6
4 carbon atoms and the alkynyl group contains from 2 to 6 carbon atoms .
- 1 3. The method of claim 2, wherein the chlorinated hydantoin is
2 monochlorodimethylhydantoin, dichlorodimethylhydantoin or a mixture thereof.
- 1 4. The method of claim 1, wherein the chlorinated hydantoin is contained in a
2 solution.
- 1 5 The method of claim 1, wherein the chlorinated hydantoin is contained in an
2 aqueous slurry.
- 1 6. The method of claim 1, wherein the chlorinated hydantoin is contained in a solid.
- 1 7. The method of claim 5, wherein the solid contains one or more chlorinated
2 hydantoins.
- 1 8. The method of claim 6, wherein the solid additionally contains one or more non-
2 chlorinated hydantoins.
- 1 9. The method of claim 1, wherein the performance additives are dispersants, scale
2 control agents, corrosion inhibitors, surfactants, biocides, cleaning agents and mixtures thereof.
- 1 10. The method of claim 1 wherein the aqueous system is a cooling water system, a
2 pulp or papermaking system, an air washer system, an agricultural potable and drainage

- 3 system, a food preparation or cleaning system, an oil industry system, a potable water system, a
4 household water-related system, or an institutional "water-related" system.

ABSTRACT

The present invention provides a method for the removal of biofilms in an aqueous system. The method involves adding one or more chlorinated hydantoins, such as dichloro and monochlorodialkylhydantoin to the aqueous system.

Customer No.:



07278

PATENT TRADEMARK OFFICE

Docket No.: 5408/OJ111

**DECLARATION
AND POWER OF ATTORNEY
Original Application**

As a below named inventor, I declare that the information given herein is true, that I believe that I am the original, first and sole inventor if only one name is listed at 1 below, or a joint inventor if plural inventors are named below, of the invention entitled:

METHOD FOR REMOVAL OF BIOFILM

which is described and claimed in:

☒ the attached specification or

☐

the specification in application Serial No.
Not Yet Assigned, filed Herewith
(for declaration not accompanying appl.)

that I do not know and do not believe that the same was ever known or used in the United States of America before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to this application, or in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to this application, that I acknowledge my duty to disclose information of which I am aware which is material to patentability in accordance with 37 CFR §1.56. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I hereby claim the priority benefits under 35 U.S.C. §119 of any application(s) for patent or inventor's certificate listed below. All foreign applications for patent or inventor's certificate on this invention filed by me or my legal representatives or assigns prior to the application(s) of which priority is claimed are also identified below.

PRIOR APPLICATION(S), IF ANY, OF WHICH PRIORITY IS CLAIMED

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COUNTRY APPLICATION NO. DATE OF FILING

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07278

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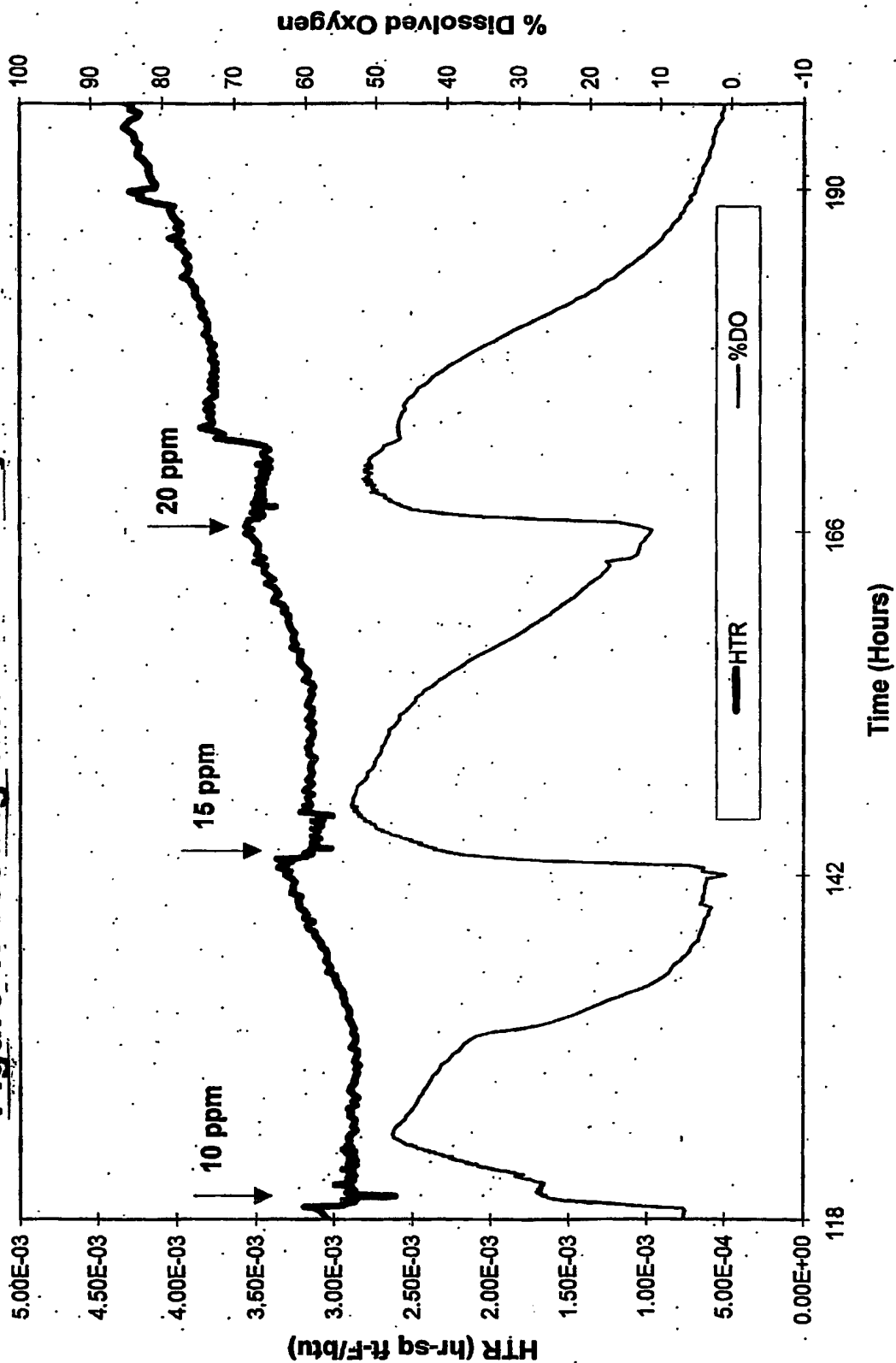
I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 1: _____ DATED: _____

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SIGNATURE OF INVENTOR 3: _____ DATED: _____

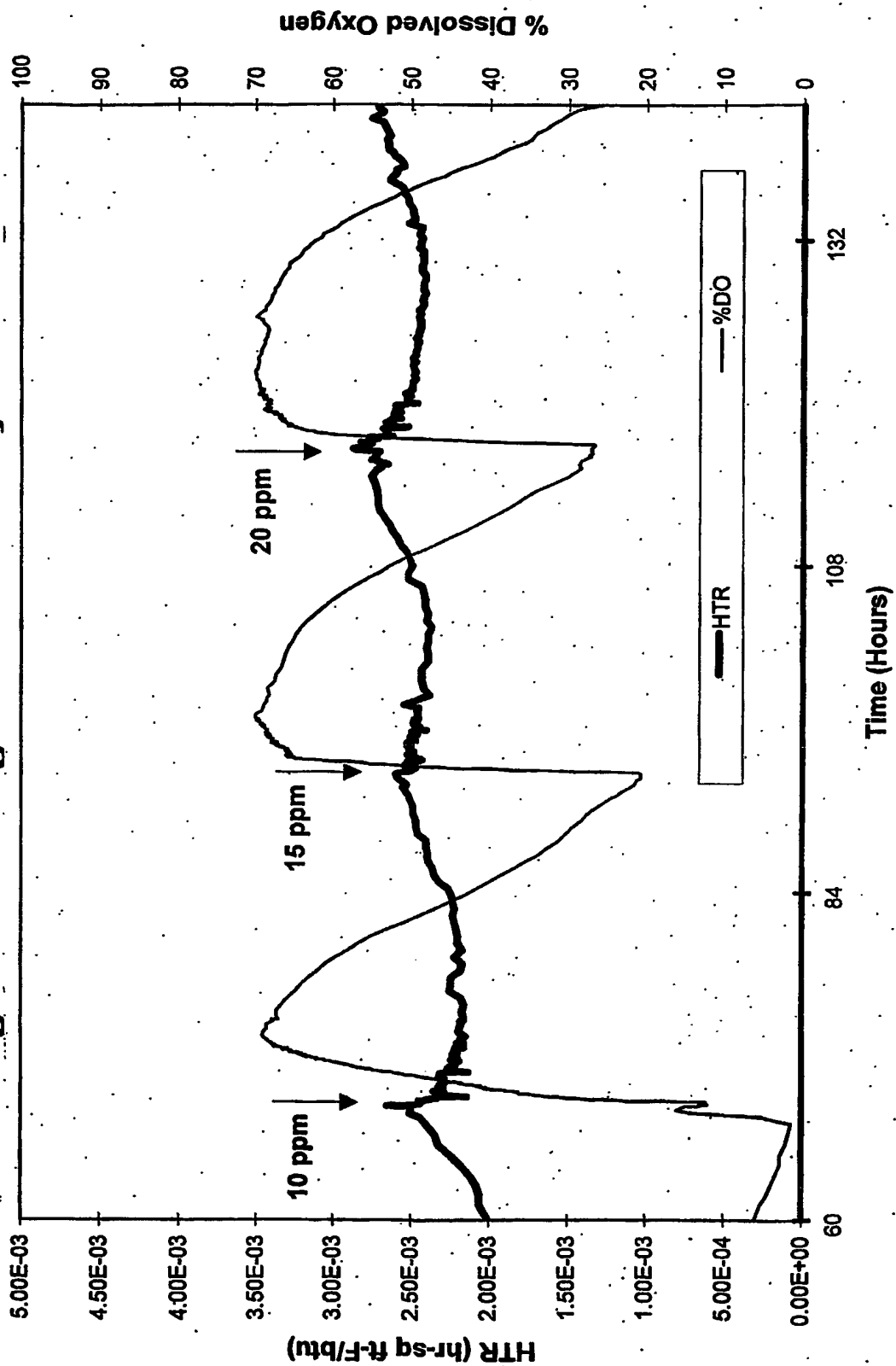
Figure 1. Testing biocidal efficacy of NaOCl



INVENTOR: Michael L. LUBENSKY, et al
 TITLE: METHOD FOR REMOVAL OF BIOMASS
 HEET: 1 OF 5
 ATTORNEY: Ben L. Lewis - (212) 571-7700
 SERIAL NO.: Not Yet Assigned
 FILE NO.: 3408/0111
 XPRESS MAIL
 DATE: _____

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Figure 2. Testing biocidal efficacy of NaOBr



ENTOR: Michael L. LUDENSKY, et al
 E: METHOD FOR REMOVAL OF BIOFILM
 ET: 2 OF 3
 Y: Ben J. Lewis - (212) 512-7700
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 E NO.: 5408/0111
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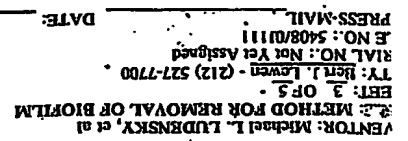
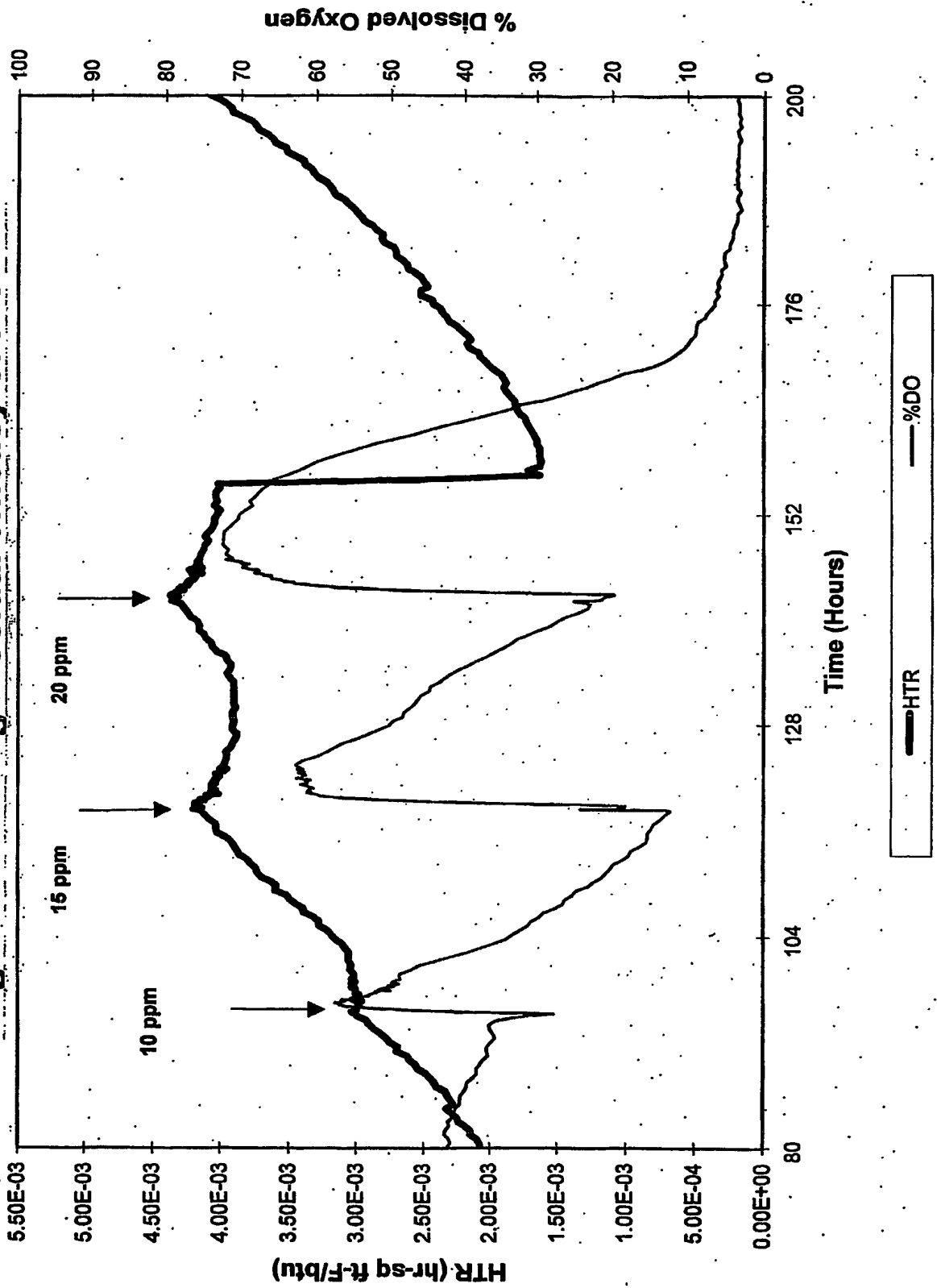


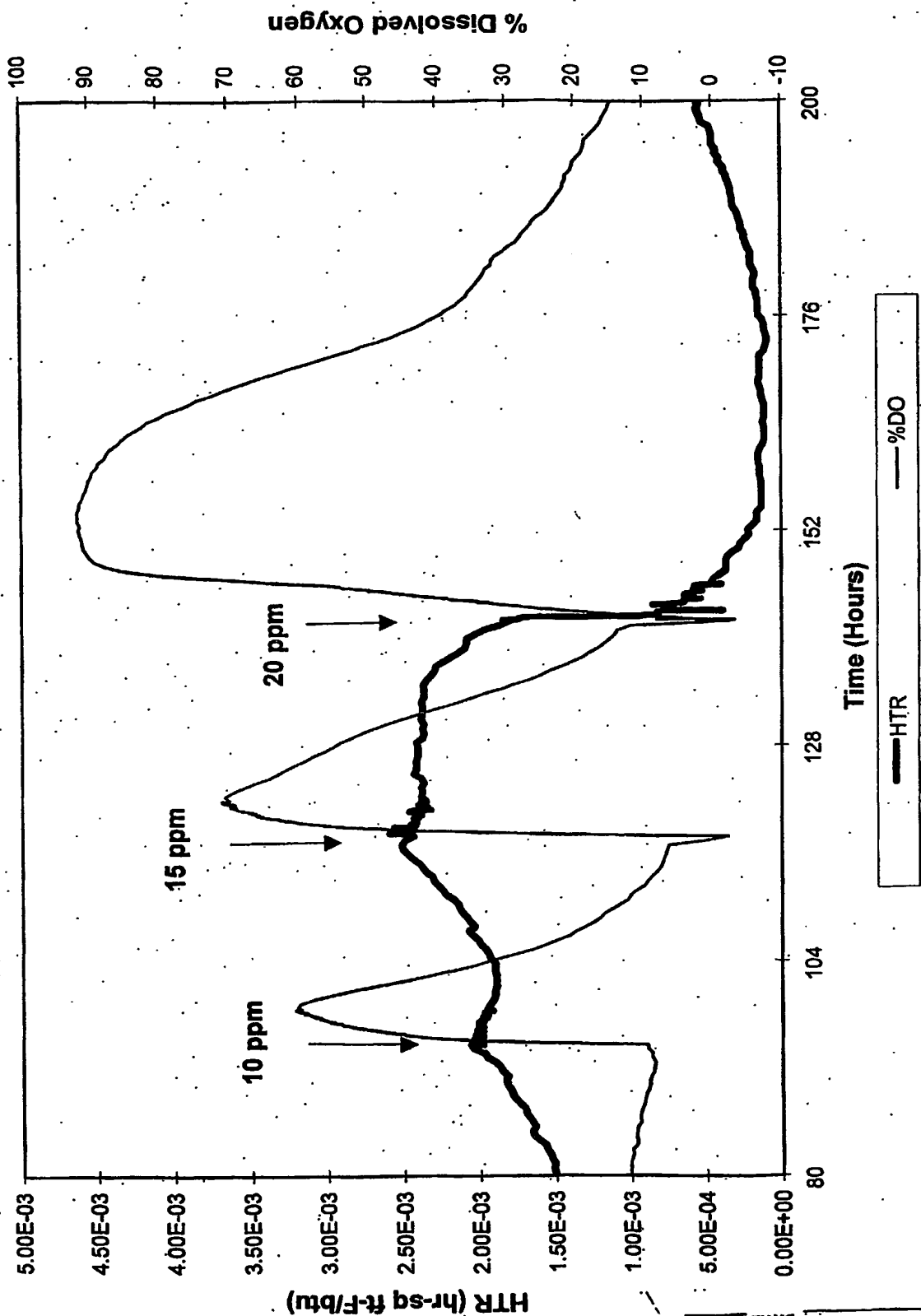
Figure 4. Testing biocidal efficacy of MCDMH



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Figure 5. Testing biocidal efficacy of DCDMH



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 3 NO.: 5408/0111
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